

WHAT IS CLAIMED IS:

1. A method of making a sensor for detecting biological recognition events comprising:

combining a biotinylated fluorescent polymer and a biotin-binding protein
5 in aqueous solution to form a complex, wherein the complex comprises free biotin-binding sites.

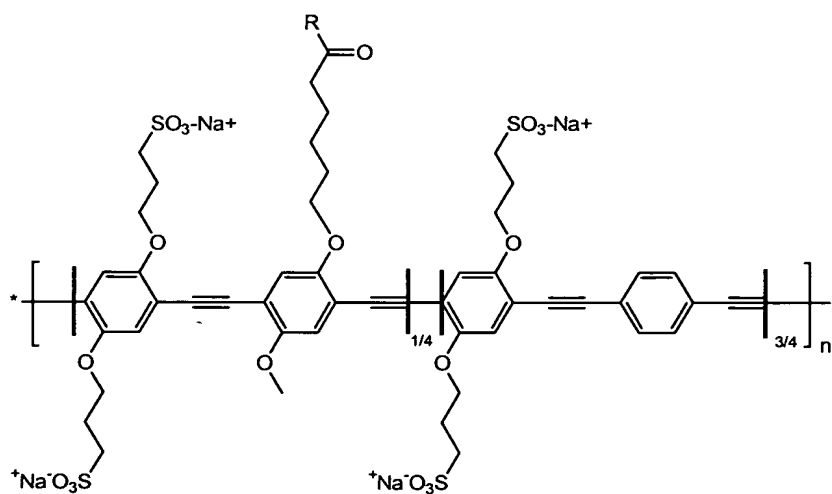
2. The method of Claim 1, further comprising:

combining a biotinylated fluorescent protein with the biotinylated
fluorescent polymer and the biotin-binding protein.

10 3. The method of Claim 2, wherein the fluorescent protein is phycoerythrin or phycobilisome.

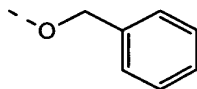
4. The method of Claim 1, further comprising disposing the complex onto the surface of a solid support.

15 5. The method of Claim 1, wherein the fluorescent polymer comprises a repeating unit represented by the following general formula:

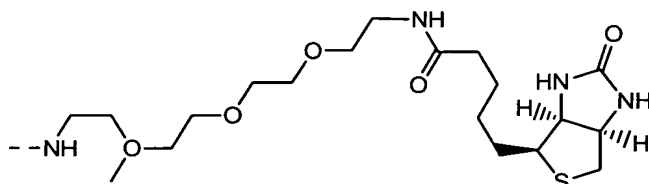


wherein n is a positive integer; and

wherein the substituent "R" is represented by the formula:



and/or the formula:



5

6. The method of Claim 4, wherein the solid support comprises a microsphere, a nanoparticle or a bead.

7. The method of Claim 4, wherein the surface of the solid support comprises a functional group selected from the group consisting of ammonium functional groups, carboxylate functional groups, charged reactive groups, and neutral reactive groups.

10

8. The method of Claim 1, wherein the biotin binding protein is selected from the group consisting of avidin, streptavidin, and neutravidin.

15

9. The method of Claim 1, the method further comprising:
adding to the solution a biotinylated bioconjugate comprising a nucleotide sequence, a peptide nucleic acid sequence or a polypeptide sequence;
wherein the biotinylated bioconjugate binds to the complex.

10. The method of Claim 9, wherein the biotinylated bioconjugate comprises a polynucleotide or peptide nucleic acid sequence and wherein the

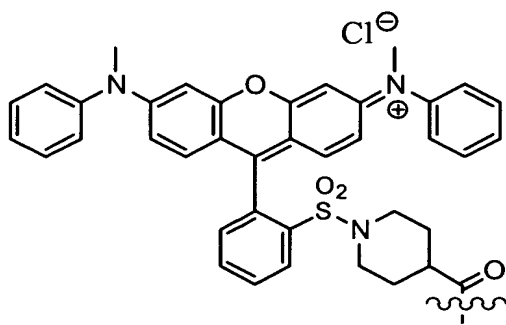
biological recognition event is nucleic acid hybridization of the polynucleotide sequence or peptide nucleic acid of the biotinylated bioconjugate to a target analyte.

11. The method of Claim 9, wherein the biotinylated bioconjugate
5 comprises a polypeptide sequence and a quencher, wherein the quencher is capable of amplified super-quenching of the fluorescent polymer and wherein the biological recognition event is enzyme induced cleavage of the polypeptide sequence.

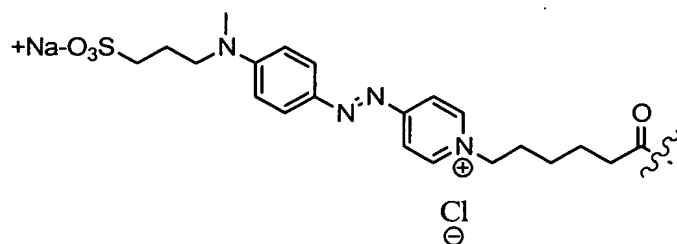
12. The method of Claim 10, further comprising:
10 adding a second bioconjugate comprising a quencher and a polynucleotide or peptide nucleic acid sequence to the solution, wherein the quencher is capable of amplified super-quenching of the fluorescent polymer and wherein the polynucleotide or peptide nucleic acid sequence of the second bioconjugate is capable of hybridizing to the polynucleotide or peptide nucleic acid sequence of the
15 biotinylated bioconjugate.

13. The method of Claim 12, wherein the polynucleotide or peptide nucleic acid sequence of the second bioconjugate is complementary to the polynucleotide or peptide nucleic acid sequence of the biotinylated bioconjugate.

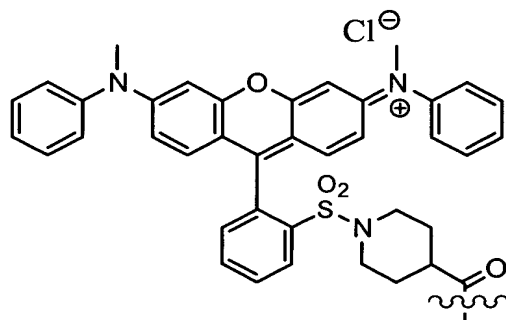
14. The method of Claim 11, wherein the quencher has a structure
20 represented by the following formula:



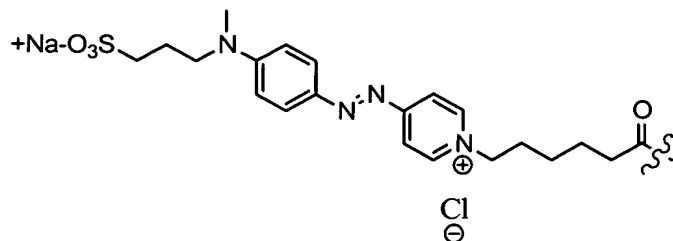
or by the formula:



15. The method of Claim 12, wherein the quencher has a structure represented by the following formula:



or by the formula:



16. A sensor for detecting biological recognition events comprising:
a complex of a biotinylated fluorescent polymer and a biotin binding
protein, wherein the complex comprises free biotin binding sites.

5 17. The sensor of Claim 16, further comprising:

a solid support:

wherein the complex is disposed on a surface of the solid support.

18. The sensor of Claim 16, further comprising:

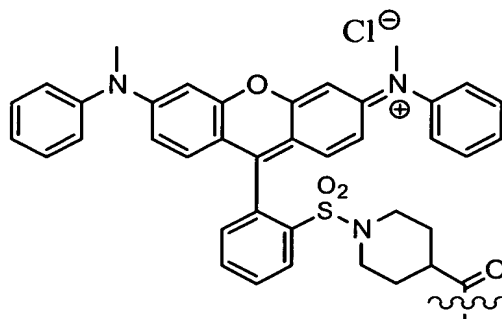
a biotinylated bioconjugate comprising a polynucleotide sequence, a
10 peptide nucleic acid sequence or a polypeptide sequence;

wherein the biotinylated bioconjugate is bound to the complex.

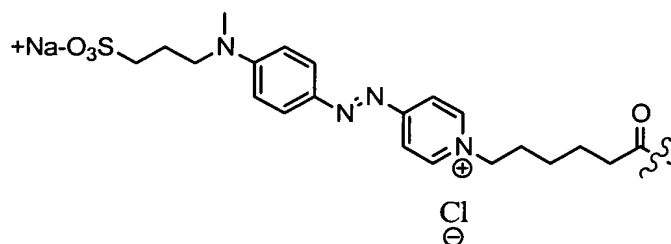
19. The sensor of Claim 18, wherein the biotinylated bioconjugate
comprises a polynucleotide sequence and wherein the biological recognition event
is nucleic acid hybridization of the polynucleotide or peptide nucleic acid sequence
15 of the biotinylated bioconjugate to a target analyte.

20. The sensor of Claim 18, wherein the biotinylated bioconjugate
comprises a polypeptide sequence and a quencher, wherein the quencher is capable
of amplified super-quenching of the fluorescent polymer and wherein the
biological recognition event is enzyme induced cleavage of the polypeptide
20 sequence.

21. The sensor of Claim 20, wherein the quencher has a structure represented by the following formula:



or by the formula:



22. The sensor of Claim 16, wherein the biotin binding protein is selected from the group consisting of avidin, streptavidin, and neutravidin.

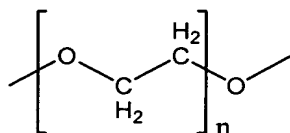
23. The sensor of Claim 17, wherein the solid support comprises a microsphere, a nanoparticle or a bead.

24. The sensor of Claim 17, wherein the surface of the solid support comprises a functional group selected from the group consisting of ammonium functional groups, carboxylate functional groups, charged reactive groups, and neutral reactive groups.

25. The sensor of Claim 17, wherein the complex further comprises:
 a biotinylated bioconjugate comprising a ligand and a biotin moiety
 conjugated to first and second locations on a tether;
 wherein the ligand comprises a quencher moiety;
 5 wherein the quencher moiety is capable of amplified super-quenching of the
 fluorescent polymer;
 wherein the ligand is capable of taking part in a biological recognition
 event; and
 wherein the portion of the tether between the first and second locations has
 10 a length and a flexibility such that occurrence of the biological recognition event
 results in separation of the quencher from the surface of the solid support with a
 concomitant increase in fluorescence.

26. The sensor of Claim 25, wherein the ligand comprises a polypeptide
 sequence.

15 27. The sensor of Claim 25, wherein the portion of the tether between the
 first and second locations comprises a repeating unit represented by the chemical
 formula:



wherein n is a positive integer.

20 28. The sensor of Claim 27, wherein n is from 70 to 80 inclusive.

29. The sensor of Claim 27, wherein n is 75.

30. The sensor of Claim 25, wherein the tether has a length of at least 250

Å in a fully extended conformation.

31. The sensor of Claim 16, further comprising:

a biotinylated fluorescent protein;

5 wherein the biotinylated fluorescent protein forms a complex with the biotinylated fluorescent polymer and the biotin-binding protein.

32. The sensor of Claim 31, wherein the fluorescent protein is phycoerythrin or phycobilisome.

33. A method of detecting the presence and/or amount of a target analyte in a sample comprising:

10 combining the sample with;

a biotinylated bioconjugate comprising a polynucleotide sequence, a peptide nucleic acid sequence or a polypeptide sequence; and
a sensor as set forth in Claim 16.

34. The method of Claim 33, wherein the biotinylated bioconjugate
15 comprises a polynucleotide or peptide nucleic acid sequence, the method further comprising:

combining the sample with a second bioconjugate comprising a quencher and a polynucleotide or peptide nucleic acid sequence;

20 wherein the quencher is capable of amplified super-quenching of the fluorescent polymer;

wherein the polynucleotide or peptide nucleic acid sequence of the second bioconjugate is capable of hybridizing to the polynucleotide or peptide nucleic acid sequence of the biotinylated bioconjugate;

and wherein the target analyte comprises a polynucleotide sequence which

is capable of hybridizing to the polynucleotide or peptide nucleic acid sequence of either the biotinylated bioconjugate or the second bioconjugate.

35. The method of Claim 34, wherein the polynucleotide or peptide nucleic acid sequence of the second bioconjugate is complementary to the polynucleotide or peptide nucleic acid sequence of the biotinylated bioconjugate.

36. The method of Claim 33, wherein the biotinylated bioconjugate comprises a polypeptide sequence and further comprises a quencher, wherein binding of the biotinylated bioconjugate to the complex quenches the fluorescence of the fluorescent polymer and wherein the target analyte is an enzyme capable of cleaving the polypeptide sequence.

37. A method of detecting the presence and/or amount of a target analyte in a sample comprising:

combining the sample with a sensor as set forth in Claim 25.

38. The method of Claim 37, wherein the ligand comprises a polypeptide sequence.

39. The method of Claim 37, wherein the target analyte is selected from the group consisting of a spore, a cell, a bacteria or a virus.

40. A sensing system for detecting biological recognition events comprising:

a sensor as set forth in Claim 25; and

a second solid support comprising a plurality of target moieties disposed on the surface thereof, wherein the ligand of the biotinylated bioconjugate interacts with the target moieties such that the quencher is separated from the fluorescer thereby increasing the fluorescence of the fluorescent polymer.

41. The sensing system of Claim 40, wherein the ligand comprises a polypeptide sequence.

42. The sensing system of Claim 40, wherein the second solid support is a microsphere, a nanoparticle or a bead.

5 43. A method of detecting the presence and/or amount of a target analyte in a sample comprising:

 combining a sensing system as set forth in Claim 40 with the sample;

 wherein the target analyte can recognize and interact with the ligand and

 wherein interaction of the target analyte with the ligand results in a decrease in
10 fluorescence.

44. The method of Claim 43, wherein the ligand comprises a polypeptide and wherein the biological recognition event comprises the interaction of the polypeptide of the ligand with a target analyte comprising a polypeptide.

45. The method of Claim 34 comprising:

15 incubating the sample with the biotinylated bioconjugate and the second bioconjugate; and

 adding the sensor to the incubated sample.

46. The method of Claim 34, wherein the sensor and the biotinylated bioconjugate are combined such that the biotinylated bioconjugate complexes to
20 the sensor, the sample is subsequently incubated with the sensor/biotinylated bioconjugate complex, and the second bioconjugate is subsequently added to the incubated sample.

47. The method of Claim 46, wherein the nucleotide sequence of the target analyte comprises a double-stranded nucleic acid, the method further comprising;

heating the incubated sample in the presence of the second bioconjugate to a temperature sufficient to melt double-stranded nucleic acid in the sample; and

cooling the sample to allow duplex formation;

wherein duplex formation between target analyte present in the sample and the second bioconjugate results in an increase in fluorescence.

48. The method of Claim 46, wherein the biotinylated bioconjugate comprises a peptide nucleic acid sequence.

49. A sensor for detecting a target biological species comprising:

a bacterial spore or virus comprising a plurality of ligands for a receptor on a surface thereof;

a fluorescent polymer or fluorescent polymer complex disposed on a surface of the bacterial spore or virus; and

a plurality of bioconjugates comprising a quencher conjugated to a receptor for the ligand, wherein the receptor and ligand interact and wherein the interaction of the receptor and ligand results in amplified super-quenching of the fluorescence of the fluorescent polymer.

50. A method of detecting the presence and/or amount of a target analyte in a sample comprising:

incubating the sample with a sensor as set forth in Claim 49;

wherein target analyte in the sample recognizes and interacts with the receptor and wherein interaction of target analyte in the sample and the receptor results in an increase in fluorescence.

51. The method of Claim 50, wherein the target analyte comprises a bacterial spore or a virus comprising a plurality of ligands for the receptor on a

surface thereof.

52. The sensor of Claim 17, wherein the biotinylated bioconjugate comprises a polynucleotide or peptide nucleic acid sequence and wherein the biotinylated bioconjugate further comprises a quencher capable of amplified
5 superquenching of the fluorescent polymer, wherein the polynucleotide sequence is located between the quencher and the biotin on the biotinylated bioconjugate.

53. A method of detecting the presence and/or amount of a target analyte in a sample comprising:

combining the sample with a sensor as set forth in Claim 52;
10 wherein the target analyte comprises a polynucleotide sequence capable of hybridizing to the polynucleotide or peptide nucleic acid sequence of the biotinylated bioconjugate and wherein hybridization results in increased separation of the quencher from the surface of the solid support and a concomitant increase in fluorescence.

15 54. The method of Claim 6, wherein the solid support comprises a silica or latex microsphere.

55. The sensor of Claim 23, wherein the solid support comprises a silica or latex microsphere.

56. The sensing system of Claim 40, wherein the surface of the second
20 solid support comprises a functional group selected from the group consisting of ammonium functional groups, carboxylate functional groups, charged reactive groups, and neutral reactive groups.

57. The sensing system of Claim 42, wherein the second solid support comprises a silica or latex microsphere.